Use of stable isotope signatures in the macroalga *Ecklonia maxima* and the filter-feeder *Mytilus galloprovincialis* to determine the extent of sewage dispersal from the Green Point Outfall, South Africa

Mark Digby Cyrus

Honours 2007

Abstract

Over the last decade natural abundances of stable isotopes ^{13}C and ^{15}N have been used to investigate the transport pathways of nutrients within aquatic ecosystems, and have contributed new understanding and knowledge to many aspects of ecology, which include tracking the spatial extents of nutrient discharges. In order to assess the uptake of nutrients from the Green Point sewage outfall in Cape Town South Africa, we selected a primary producer, the kelp *Ecklonia maxima* (for dissolved nutrients) and a filter-feeding organism, the blue mussel *Mytilus galloprovincialis* (for organic particulates).

It was hypothesized that biota within close proximity of the outfall would have isotopic values (signatures) which were closer to the values recorded for sewage and that this signature would become weaker (more positive) with increasing distance from the outfall as the sewage would be diluted and dispersed.

Results from our study indicated that the stable isotope abundances of biota near the outfall were significantly affected by sewage. *Ecklonia maxima* situated on the outfall itself had a $\delta^{15}N$ of 1.2±2.3 ‰, which was extremely depleted relative to our control (8.3±1.1‰), collected at Mauritzbaai. The $\delta^{15}N$ recorded for raw sewage (0.4±0.4‰) was very similar to that of *Ecklonia maxima* situated on the outfall. *Mytilus galloprovincialis* were also affected by sewage organic matter however although the effects on the stable isotope abundances were less pronounced as they were relying on more than one source of food. The isotopic values recorded in this study demonstrated that sites which were in close proximity to the outfall and even those just within the study area were contaminated by sewage effluent. From this study we conclude that the $\delta^{15}N$ signatures of *Ecklonia maxima* and *Mytilus galloprovincialis* in marine environments can provide a useful means of tracing sewage dispersal in well-mixed ocean conditions, where conventional methods may have failed to reveal the extent of dispersal.

KD CYRU

Introduction

Throughout the world, increasing coastal urbanization has resulted in a steady increase in the amount of sewage being discharged into the marine environment (Gartner *et al.* 2002). Sewage discharge by marine outfall has been widely practiced by coastal urban developments due to its relatively low input and running costs. This type of disposal is based on the assumption that a well-designed outfall can, through dilution of the effluent, discharge its waste safely into the sea. This might be applicable to pathogens or physiologically toxic substances but is incorrect as far as organic and nutrient loading are concerned (Monteiro 1993). When organic-rich sewage is discharged into the ocean, dilution does not reduce the extent to which effluent affects the carbon and nitrogen cycle within the water column (Monteiro 1993). Sewage effluent therefore contributes significantly to the amount of anthropogenically-derived dissolved nutrient and particulate organic matter discharged into the marine environment (Piola *et al.* 2006). Understanding the fate and processing of these anthropogenic nutrients is crucial for water-quality management, as well as for assessing the assimilation capacity of coastal zones (Savage & Elmgren, 2004).

In the past, studies assessing the distribution and effects of sewage discharge have been hampered mainly by the fact that the chemical and physical parameters measured such as salinity, dissolved nutrients, bacteria and organic matter composition are characteristic of not only domestic sewage but also industrial effluents, diffuse source inputs such as storm waters as well as natural fluxes (Monteiro 1993). In order to overcome these difficulties a range of tracers and biomarkers have been used (Costanzo *et al.* 2001). The most successful of these has been coprostanol (Bartlett *et al.* 1985) a biomarker of human sewage. Although it is highly specific it has two major draw backs. The first is that it does not follow the same pathways and rates as biogenic carbon and nitrogen, which is important because it is not able to provide a direct assessment of the carbon and nitrogen loading within a system (Monteiro 1993). The second drawback is that its isolation and measurement are analytically complex (Monteiro 1993). All of the above techniques and variables provide us with useful tools for determining the physical extent of sewage effluent; however they provide little insight into the biological uptake and impact of sewage nutrients (Costanzo *et al.* 2001).

During the past decade the use of the natural abundances of ^{13}C and ^{15}N to investigate the transport pathways of nutrients within aquatic ecosystems has contributed new understanding and knowledge to many aspects of ecology, including the spatial extents of nutrient discharge (Costanzo *et al.* 2005). This is because sewage-derived nutrients and organic matter have terrestrial carbon and nitrogen isotopic signatures, which are sufficiently distinct from autochthonous marine primary production. This means that their dispersal and uptake by species within surrounding waters can be monitored (Waldron *et al.* 2001). By determining the isotopic values of the two end members (sewage and uncontaminated sea water) we can use source proportionality to estimate the contribution of sewage nutrient to the affected marine populations (Rogers, 2003).

During the sewage treatment process, bacteria digest nitrogen and carbon, thereby reducing their concentrations within the effluent and helping to minimize negative environmental impacts. During this process the bacteria have an enzymatic preference for the lighter isotopes (^{14}N & ^{12}C) as these are easier to metabolise (Heaton, 1986). The remaining nitrogen and carbon within the effluent is therefore enriched with the heavier isotopes (^{15}N & ^{13}C). Plants and organisms which utilize these nutrients will also contain more of the heavier isotopes and therefore have more similar isotopic values to sewage, relative to natural populations. These differences can therefore be monitored to track sewage distributions (Costanzo *et al.* 2005). Previous research has shown that sewage effluent has carbon and nitrogen isotope ratios of around -23.5 ‰ for $\delta^{13}C$ and between 1.8 ‰ and 2.5 ‰ for $\delta^{15}N$ (Sweeney *et al.*1980; Spies *et al.* 1989; Van Dover *et al.* 1992; Rogers. 2003).

Members of a food-web utilizing sewage-derived nutrients and organic matter in their diet would therefore be expected to have more negative $\delta^{13}C$ and $\delta^{15}N$ values than the same species of organism using only marine-produced nutrients (Rogers. 2003).

Carbon isotopes

The carbon isotope ratio of a consumer reflects its carbon assimilation, with enrichment in ^{13}C caused by biosynthesis processes that preferentially use the lighter ^{12}C isotope (Rogers 2003). This means that the $\delta^{13}C$ values of consumers are usually more positive than their food. Peterson & Howarth (1987), however, found that a decrease in $\delta^{13}C$ values within organisms occurred with increasing size and age. Differences in $\delta^{13}C$ values between terrestrial and marine produced organic matter is dependent on carbon source contribution. Atmospheric CO_2 has a $\delta^{13}C$ value of -8.8 ‰; however marine organisms use dissolved inorganic carbon (DIC) and/or seawater bicarbonate resulting in average $\delta^{13}C$ values of -18.0 ‰, compared to mean $\delta^{13}C$ values of -26.5 ‰ for terrestrial C_3 plants (Vogel *et al.* 1978; Ceilinig 1997).

Nitrogen isotopes

Sewage particulate matter has a more negative nitrogen stable isotope ratio than marine plants (up to 8‰ lower, Spies *et al.* 1989). In open systems biosynthetic processes result in decreased $\delta^{15}N$ values, as vegetation fractionates the available nutrients and preferentially takes up the lighter isotope (^{14}N). Fractionation of nitrogen isotopes between trophic levels is usually greater than for carbon, resulting in up to a 3‰ increase in δ^{15} Nitrogen. Nitrogen assimilation is also dependent on the nitrogen compound available for uptake by plants at the base of the food chain, and therefore allows us to distinguish between marine and terrestrial food sources (Schoeninger & Wada 1984).

The objectives of this study were to determine whether it is possible to track the spatial and temporal distribution and uptake patterns of sewage from the Green Point Sewage Outfall, in Cape Town, using stable carbon and nitrogen isotopes

from two very different bio-indicators. The two bio-indicators were selected to represent the uptake of the two different forms of nutrients being discharged: those being dissolved nutrients and particulate organic matter. To assess the uptake of dissolved nutrients we selected a primary macroalgal producer, the kelp *Ecklonia maxima,* because macroalgae take up nutrients directly from the water column and a perennial kelp therefore demonstrates the long-term availability of nutrients within a contaminated area. Variations of $\delta^{15}N$ in naturally-occurring marine plants adjacent to sewage outfalls provide a technique for detecting and mapping the geographical extent of biologically-available sewage nitrogen.
To track the distribution and uptake of particulate organic matter we chose to use a filter-feeding organism, the blue mussel (*Mytilus galloprovincialis*). The reason for choosing a filter feeder is that they assimilate particulate organic matter into their tissue at levels significantly above those found in the surrounding environment, due to the large volumes of water they are able to filter (Piola *et al.* 2006).

The primary objective of this study is to develop a method of mapping the distribution of discharged sewage as well as allow delineation of the influences from sewage-derived organic carbon and nitrogen compared to other inputs within the proximity of the outfall. It is hypothesized that biota within close proximity of the outfall will have isotopic values (signatures) which are closer to the values recorded for sewage relative to communities situated further from the outfall. We also hypothesize that this signature will become weaker (more positive) with increasing distance from the outfall as the sewage is diluted and dispersed.

Material & Methods

Green Point Outfall

The Green Point Sewage Outfall, in Cape Town, serves to discharge raw domestic sewage from the central Cape Town, Sea Point and Green Point areas. Together it is estimated that this outfall provides for a combined population of at least 250

000 people (Fijen & Marsden 1992). The outfall is 1700m in length and discharges sewage at a depth of 28m, at an average rate of approximately 30 Mega liters per day, or 340 liters per second (Fijen & Marsden 1992). Pretreatment of the effluent consists of coarse screening, maceration and fine screening processes. This pretreatment ensures that particles which are discharged from the outfall have a maximum size of 3mm (Fijen & Marsden. 1992). The outfall itself is located just off Mouille Point (33° 53' 58 S, 18° 23' 54 E) on the edge of Table Bay (Fig 1).

Site Description

This study involved the sampling of 7 sites located along the west coast of Cape Town on the western side of the Cape Peninsula. The study area comprised a section of coastline which was 5 km in length and extended from Bantry Bay (33° 55' 27 S, 18° 22' 36 E) to just west of Granger Bay (33° 53' 56 S, 18° 24' 26 E).

The section of Atlantic coast selected is impacted by the Benguela upwelling system, with cool seawater temperatures in summer. (Monteiro 1993). The seabed in the surrounding area is described as rocky with occasional patches of sand and sediment (Monteiro 1993).

The 7 study sites within the study area were situated both immediately inshore of the outfall and on either side of it (Fig 1). Sites 1-5 were situated to the south west of the outfall, with Site 1 situated at Bantry Bay (33° 55' 27 S, 18° 22' 36 E), Site 2 at Sunset Beach (33° 55'13 S, 18° 22' 47 E), Site 3 at Boat Bay (33° 54' 53 S, 18° 22' 36 E), Site 4 at Three Anchor Bay (33° 54' 22 S, 18° 23' 43 E) and Site 5, just south of Mouille Point (33° 54' 11 S, 18° 23' 52 E). Site 6 was situated nearest to the outfall itself which is just off Mouille Point (33° 53' 58 S, 18° 23' 54 E). Site 7 was located to the eastern side of the outfall in close proximity to Granger Bay (33° 53' 56 S, 18° 24' 26 E). Study sites were chosen at random and were aimed to represent increasing distance from the outfall (site 6) as well as different inter-tidal sites (exposed and sheltered). In order to get an accurate representation of isotopic values for *Ecklonia maxima* and *Mytilus galloprovincialis* in uncontaminated areas, samples were collected from a control site in Mauritzbaai

(32 °58'50 S, 17 ° 53'10 E) 4km outside of Jacobsbaai, which was to act as our control site for this study.

Fig 1: Satellite image of the study area showing the sampling sites 1-7.
(Google Earth 2007)

Sampling Methods

Sampling for this study was conducted on the 27 June 2007 during low tide. At each site 6 individual blue mussels (*Mytilus galloprovincialis*) and 6 kelp fronds from separate *Ecklonia maxima* individuals were collected.

During the collection of mussels, considerable efforts were made to ensure that specimens were of similar size (6-9cm) and collected from similar tidal levels. This was done because these organisms are attached to rocks within the intertidal zone and are not able to move. Therefore these sessile bio-indicators were selected to be of similar age and to be submerged for similar time intervals during tidal cycles.

In order to control for the age, reproductive and metabolic activity of kelp fronds, algal samples for this study were collected from central secondary fronds on mature specimens. Only specimens which were attached to rocks were collected as apposed to free-floating ones. The reason for this was to ensure our sample site's integrity, as the origin of floating material is unknown and therefore misrepresentative of the area where it was collected.

Sewage samples were collected from the Green Point treatment plant using a long rope and a bucket as the samples needed to be collected from out of a canal.

Phytoplankton was not sampled in this study; instead values from Bustamante & Branch (1996) were used.

Laboratory Processing

After collection all samples were cleaned in de-ionised water and gently brushed to remove any epiphytes and micro-organisms. Samples were then placed into a drying oven at a temperature of 60 °C, where they were left until they reached a constant weight. Once dry, mussel flesh was removed from the shells. All samples were then ground for 5 min using a Retsch mm200 ball mill in order to homogenize them and create a fine powder. Powdered samples were then weighed into tin cups with an accuracy of 1 microgram, using a Sartorius micro balance. Different amounts of material were used for mussel and kelp analysis as there are differences in the amounts of carbon and nitrogen contained within them. Between 0.6-0.7 mg of mussel material was required while kelp required at least 2 mg of material. Once the samples had been weighed into the tin cups, the cups were squashed to enclose the sample.

Sewage samples were filtered using a hand pump and Munktell Glass-Microfibre filter disks which had a filtering diameter of 47 mm. After filtration, filter papers were dried to a constant weight in a drying oven at a temperature of 60 °C. The sewage was then removed from the filter papers and placed into tin cups for combustion.

The Samples were then combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy), and the gases created during this combustion were then passed to a Delta Plus XP Isotope Ratio Mass Spectrometer (IRMS) (Themo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). Stable isotope values are then calculated as:

$$\delta (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

Where R is the ratio of the heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$).
The values are expressed relative to a standard, namely, atmospheric air for Nitrogen and Vienna Pee Dee Belemnite (VPDB) for Carbon. Samples with a high δ value are referred to as being "heavier" or "enriched" while samples with lower δ values are referred as being "lighter" or "depleted" relative to the heavy isotope (^{15}N or ^{13}C). The deviation from the standard is denoted by the term δ and the results expressed as parts per thousand (‰).

Data analysis

One way ANOVA's and post-hoc Tukey tests were performed on $\delta^{13}C$ and $\delta^{15}N$ values collected for both kelp (*Ecklonia maxima*) and mussels (*Mytilus galloprovincialis*) (Statistica 7).
Cluster analyses using complete linkage and Euclidean distance were created for $\delta^{13}C$ and $\delta^{15}N$ values collected for both kelp (*Ecklonia maxima*) and mussels (*Mytilus galloprovincialis*) using a community analysis package (CAP, Pisces 2002).

Results

Carbon and nitrogen stable isotopes and elemental abundances determined from kelp (*Ecklonia maxima*), mussels (*Mytilus galloprovincialis*) and milli-screened POM sewage from Green Point Outfall are presented in Table 1.

Table 1: Mean organic carbon and nitrogen content, $\delta^{15}N$ and $\delta^{13}C$ values of macro-algae (*Ecklonia maxima*) and shellfish tissue (*Mytilus galloprovincialis*) from the 7 study sites, as well as $\delta^{15}N$ and $\delta^{13}C$ values for sewage POM from Green Point outfall. (with Standard Errors).

Species	Sites	$\delta^{15}N$	$\delta^{13}C$	%N	%C
Ecklonia maxima	1	4.1±0.8	-12.5±0.8	1.6±0.07	28.8±0.9
	2	5.6±0.5	-13.2±0.8	1.5±0.08	28.6±0.9
	3	5.1±1.4	-12.8±0.6	1.8±0.09	29.8±0.9
	4	4.9±1.4	-12.0±1.1	1.7±0.10	28.9±0.9
	5	3.3±1.3	-13.8±1.4	1.6±0.11	26.8±1.1
	6	1.2±2.3	-13.0±0.8	1.6±0.12	28.3±0.9
	7	5.6±1.5	-11.9±0.8	2.0±0.10	30.4±1.0
Mytilus galloprovincialis	1	7.3±0.2	-16.0±0.3	9.4±0.7	37.9±2.1
	2	7.5±0.3	-14.9±0.2	8.8±0.7	35.2±1.7
	3	7.7±0.3	-15.7±1.0	9.9±1.1	40.2±4.1
	4	7.5±0.2	-15.6±1.0	9.3±0.9	37.3±3.6
	5	7.6±0.3	-15.7±0.9	10.1±0.8	40.8±3.4
	6	7.0±0.3	-16.1±0.9	10.3±0.8	40.6±3.6
	7	7.9±0.3	-15.4±0.8	9.5±0.9	40.6±3.7
Green Point effluent POM		±0.4	[illegible]2.8±0.2		

Sewage and sea water

Effluent collected from the Green Point sewage outfall was shown to have $\delta^{13}C$ values of -22.8 ± 0.2‰ and $\delta^{15}N$ values of 0.4±0.4‰ respectively (Table 1). Sea water from this area was not sampled as we lacked the apparatus and funding to determine $\delta^{13}C$ and $\delta^{15}N$ values. Values from the literature indicate that sea water from Houghton Bay, New Zealand has $\delta^{13}C$ values of -20.1 ± 0.1‰ and $\delta^{15}N$ values of 6.5±0.3‰ (Rodgers 1999).

Kelp

The average $\delta^{15}N$ value for *Ecklonia maxima* from our control site at Mauritzbaai was 8.3±1.1‰ (Table 1, Fig 2). Sample site 7 situated on the opposite side of the Green Point outfall and site 2 had very similar isotopic values, which were closest to those recorded at the control site (Table 1, Fig 2). Site 1, although situated far from the outfall, was shown to have more depleted values than similar sites closer to the outfall (4.1±0.8‰), with sites 3 and 4 closer to the outlet being similarly depleted (5.1±1.4 & 4.9±1.4‰) (Table 1, Fig 2). Sites 5 and 6 were the closest sites to the outfall and showed the most depleted $\delta^{15}N$ values which were 3.3±1.3 and 1.2±2.3‰ respectively (Table 1, Fig 2). Site 6 which was on the outfall had the lowest $\delta^{15}N$ values, which were on average 7.1‰ lighter than those of the control. The values recorded for this study seem to indicate that there is an increase in $\delta^{15}N$ as one gets further from the sewage outfall. This is true for all sites except for site 1.

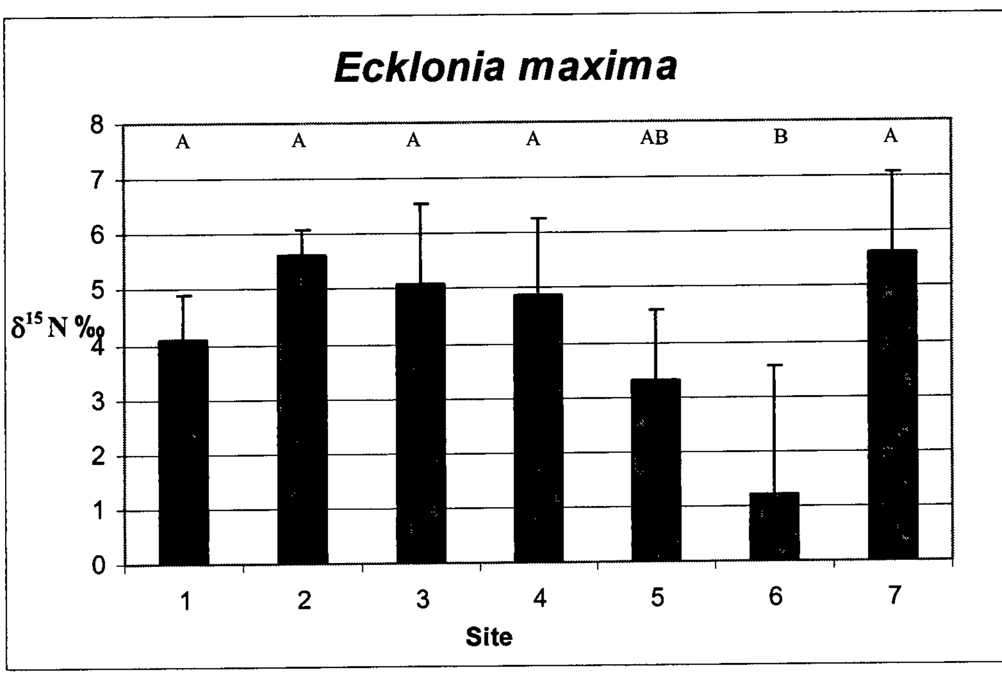


Fig 2: Mean δ^{15}N values for *Ecklonia maxima* at the 7 sampling sites within the study area. Data are means ± 1 SE. Letters (AB) indicate significant differences at the p=<0.5% level (post-hoc Tukey test).

Statistical analysis of these results showed that there were significant differences in the $\delta^{15}N$ values for *Ecklonia maxima* at all sites sampled (ANOVA, p = 0.000037). Site 6 which was located on the outfall was shown to have significantly different $\delta^{15}N$ values from all other sites except for site 5 which was the next closest site to the outfall (Tukey, $p(1:6)$= 0.019, $p(2:6)$=0.0002, $p(3:6)$=0.0008, $p(4:6)$=0.0015, $p(7:6)$=0.0002).

Carbon isotopic values of *Ecklonia maxima* sampled at our control site averaged -13.38‰. $\delta^{13}C$ values at all sample sites did not vary much, and ranged between -11.9 and -12.8‰ (Table 1, Fig 3). Site 5 was shown to have the most depleted values while site 7 had the most enriched. Statistical analysis of the results revealed that there were significant differences between sites even if they were small (ANOVA, p=0.01). A Tukey test revealed that the main differences were found between sites 5 & 4 and 5 & 7 which gave significant p values of 0.02 and 0.01 respectively.

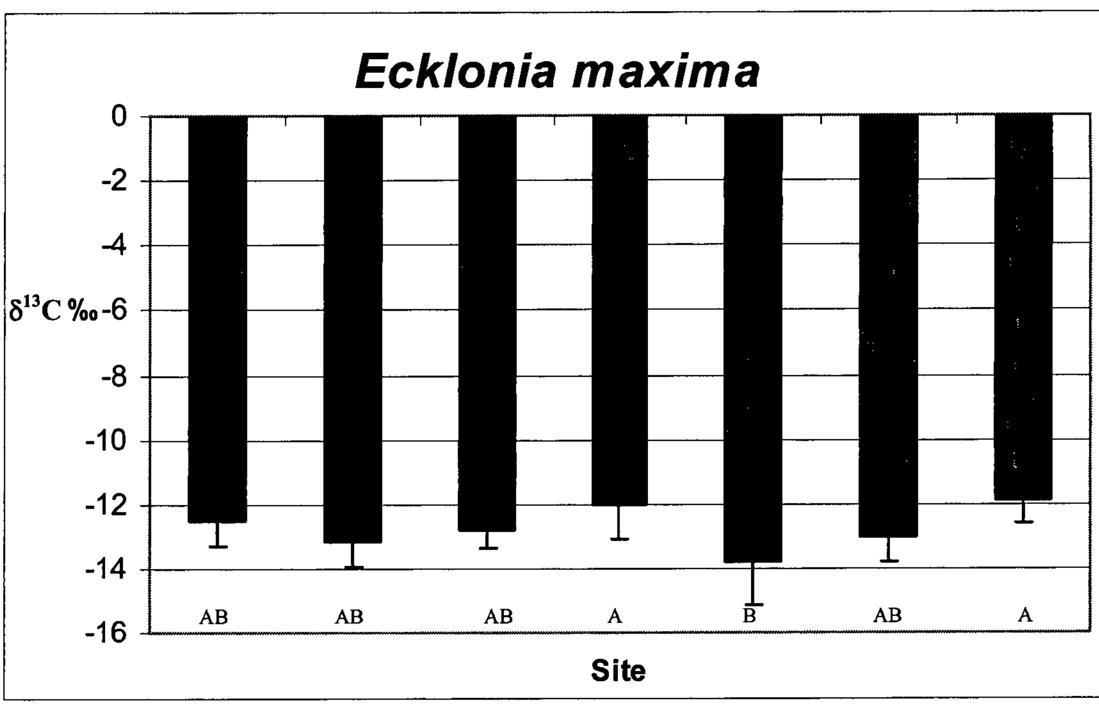
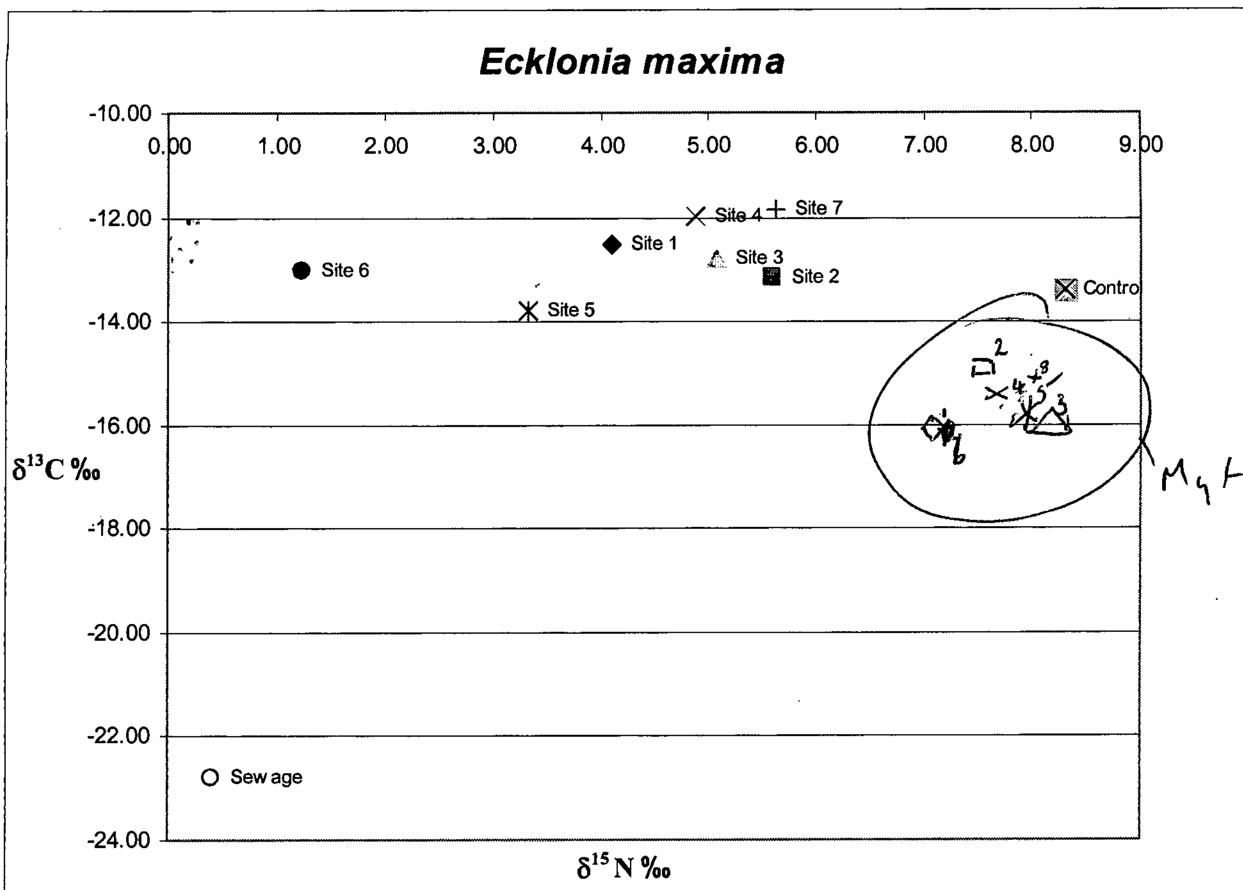


Fig 3: $\delta^{13}C$ values for *Ecklonia maxima* at the 7 sampling sites within the study area. Data are means ± 1 SE. Letters (AB) indicate significant differences at the p=<0.5% level (post-hoc Tukey test).

Sites vary greatly in terms of their $\delta^{15}N$ values while $\delta^{13}C$ values change only slightly (Fig 4). Our control site is most similar to sites 2 and 7, with $\delta^{13}C$ values similar to the rest of the sites. Site 6 shows very similar $\delta^{15}N$ values to the raw sewage sampled from the treatment station. Sites 1 and 5 also tend to show $\delta^{15}N$ values that are similar to those of raw sewage however they are not as pronounced.

Percentage (%) Nitrogen varies very little between the different sites, with all values falling between 1.5 and 2% (Fig 5). Site 7 has the highest % N while site 2 has the lowest.



Mytilus

Fig 4: A comparison between $\delta^{15}N$ and $\delta^{13}C$ of *Ecklonia maxima* from the 7 sample sites, control site (Mauritzbaai) and raw sewage sampled directly from the Green Point treatment station.

Figure 6 is a cluster analysis showing similarities between sites due to $\delta^{15}N$ values. Sites 1 and 5 are very similar and quite different from the rest of the sites. The same situation can be seen between site 6 and the sewage collected from the treatment plant as these values are distinctly different and have been grouped together separated from all other groups. Sites 2, 3, 4 and 7 are all shown to have some similarity and are grouped together. This group is also shown to have some association with sites 1 and 5 as there are slight similarities.

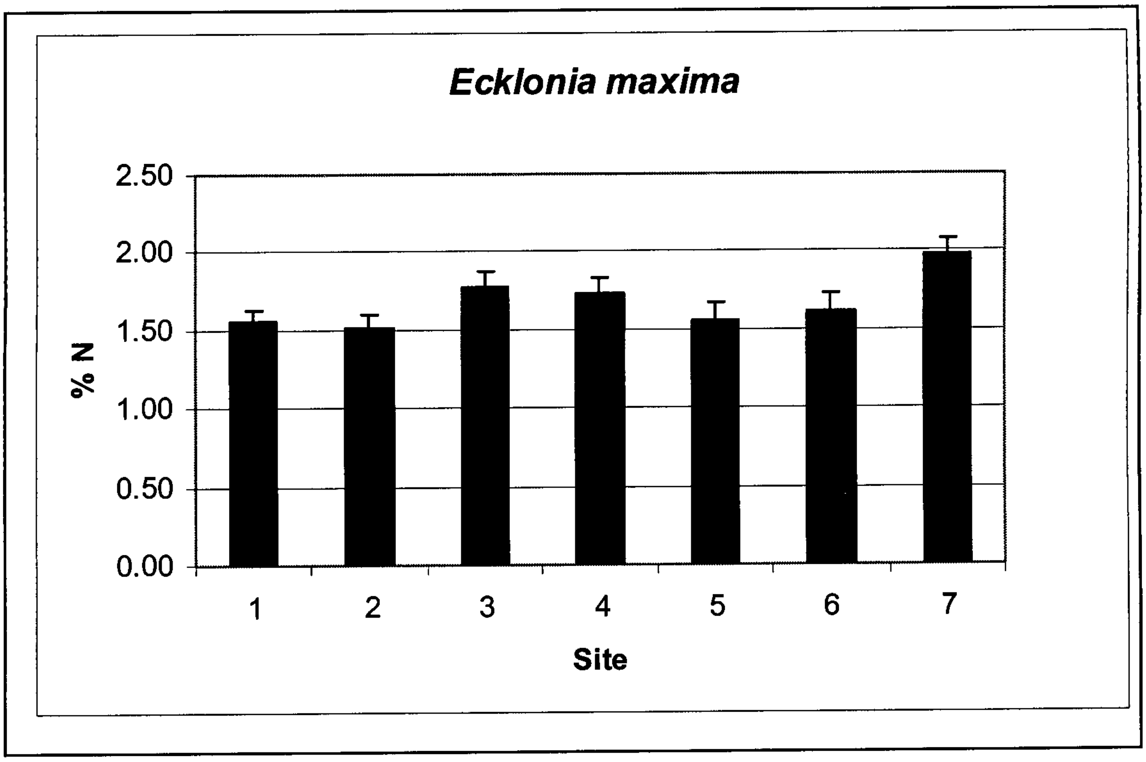


Fig 5: Average % Nitrogen of *Ecklonia maxima* at each sample site. Data are means ± 1 SE.

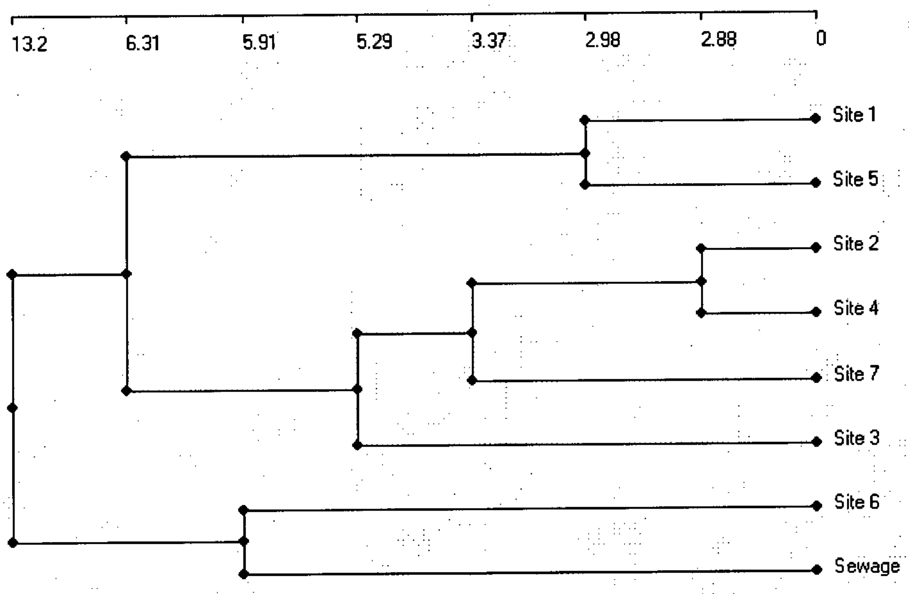


Fig 6: Cluster analysis of $\delta^{15}N$ values of *Ecklonia maxima* at sampling sites using complete linkage and Euclidean distance.

Mussels

The tissue collected from blue mussels (*Mytilus galloprovincialis*) at the 7 sample sites revealed that $\delta^{15}N$ values did not vary much between sites, with the most depleted site being 6 and the most enriched 7 (7.0±0.3 and 7.9±0.3‰) (Table 1, Fig 7). Compared with our control site (8.51±0.25‰) all sites in the study area had depleted $\delta^{15}N$ values. Although $\delta^{15}N$ values between sights only varied slightly there are definite trends in the data. Statistical analysis revealed that there was a significant difference in $\delta^{15}N$ values between sites (ANOVA, p= 0.0003). The $\delta^{15}N$ values for the mussels at the 7 sites followed a similar trend to the $\delta^{15}N$ values recorded for macro-algae, except that the trend was much less pronounced. Site 6 was significantly different from all other sites except for site 1 (Tukey, $p(2:6)=0.04$, $p(3:6)=0.001$, $p(4:6)=0.04$, $p(5:6)= 0.02$, $p(7:6)=0.0002$).

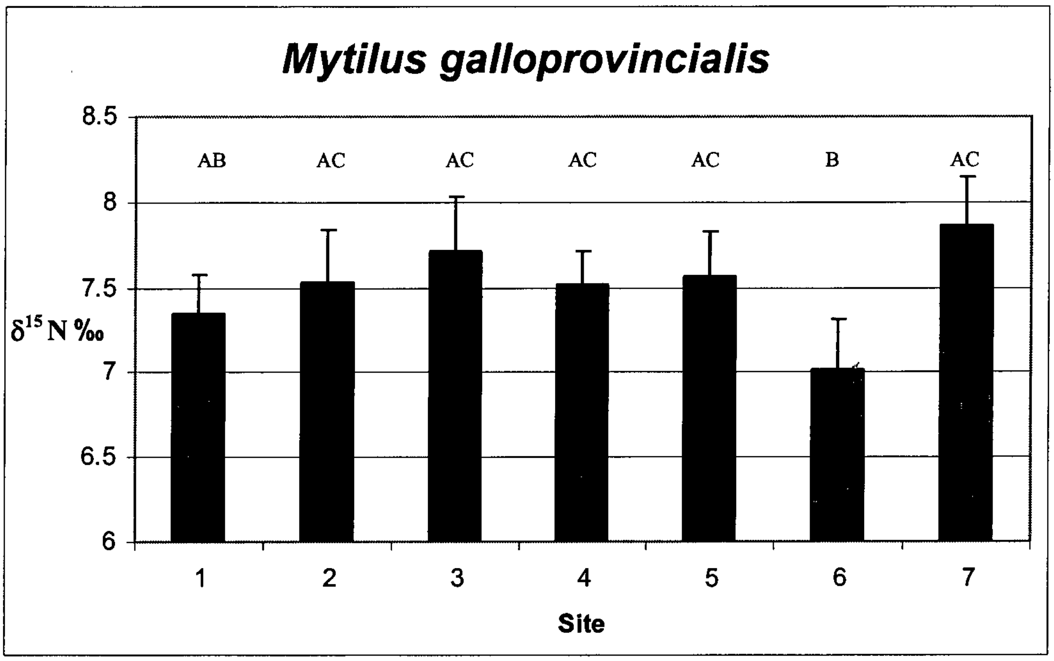


Fig 7: Bar graph representing the δ^{15} N values for *Mytilus galloprovincialis* at the 7 sampling sites within the study area. Data are means ± 1 SE. Letters (ABC) indicate significant differences at the p=<0.5% level (post-hoc Tukey test).

Carbon isotope analysis of mussel tissue revealed no significant differences in isotopic abundance between sites. Site 6 was the most depleted with an average value of 16.1±0.9‰ while site 2 had the most enriched values with an average of -

14.9±0.2‰ (Table 1, Fig 8). Compared to our control site (-15.31±0.15‰) all sites except 2 had $\delta^{13}C$ values which were more depleted.

A one-way ANOVA of this data revealed that there was a significant difference between sites (p=0.0002), with the main differences occurring between site 2 and sites 1,3,4,5 and 6 (Tukey, p(2:1)= 0.0008, p(2:3)= 0.01, p(2:4)= 0.04, p(2:5)=0.02, p(2:6)= 0.0003).

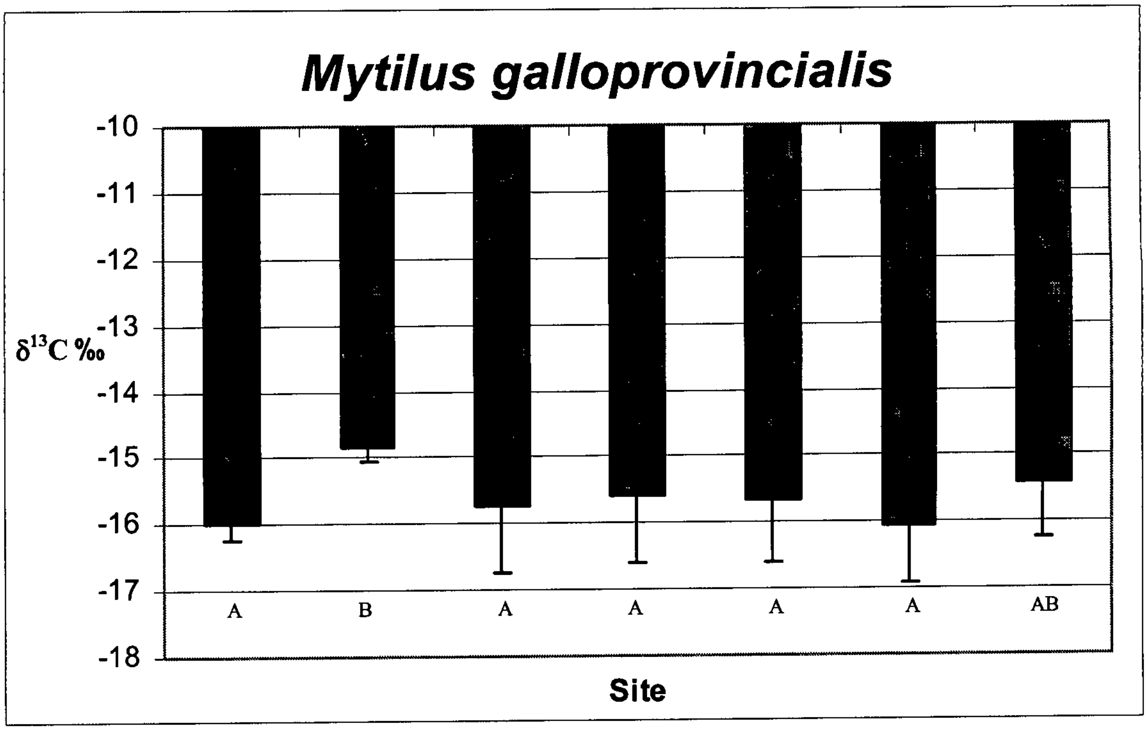


Fig 8: Bar graph representing the $\delta^{13}C$ values for *Mytilus galloprovincialis* at the 7 sampling sites within the study area. Data are means ± 1 SE.

The results from *Mytilus* are grouped more closely than those for *Ecklonia maxima,* and vary only slightly in terms of both their $\delta^{15}N$ and $\delta^{13}C$ values (Fig 9). There are still however visible trends between sites as we can see that site 6 is most similar to the values recorded for sewage, and that site 1 also has depleted $\delta^{15}N$ and $\delta^{13}C$ values similar to site 6. Our control site has similar $\delta^{15}N$ values to phytoplankton as well as *Ecklonia maxima* (control). $\delta^{15}N$ and $\delta^{13}C$ values for all sites are very similar to our *Ecklonia maxima* (control). Site 2 differs from the other sample sites in due to its enriched $\delta^{13}C$ value.

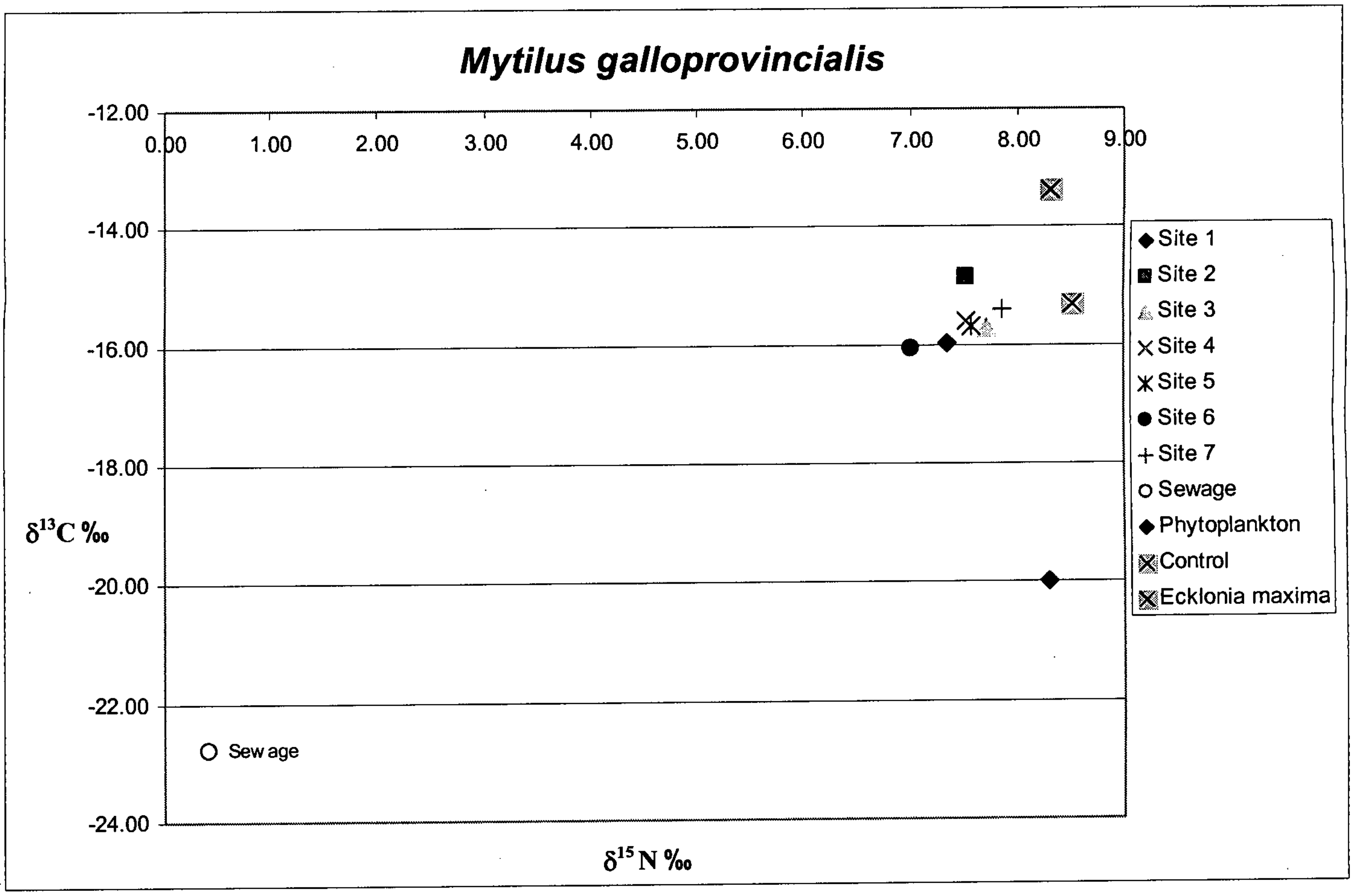


Fig 9: A comparison between $\delta^{15}N$ and $\delta^{13}C$ of *Mytilus galloprovincialis* from the 7 sample sites, control site (Mauritzbaai), phytoplankton and raw sewage sampled directly from the Green Point treatment station.

Figure 10 indicates that the percent nitrogen in *Mytilus galloprovincialis* is higher than that found in *Ecklonia maxima*, with a larger range of values (8.8±0.7 and 10.3±0.8) (Table 1, Fig 9). Sites 6 and 5 showed the highest %N within sampled mussels, while site 2 showed the lowest. Site 3 showed values that were similar to sites 6 and 5.

Figure 11 is a cluster analysis showing the similarities between sites due to $\delta^{15}N$ values, in this representation 3 distinct groups are evident, those of sites 1, 4 and 2, sites 3, 5 and 7, and site 6 and sewage. Although not such a strong association site 6 and sewage are distinctly different from the rest of the sites.

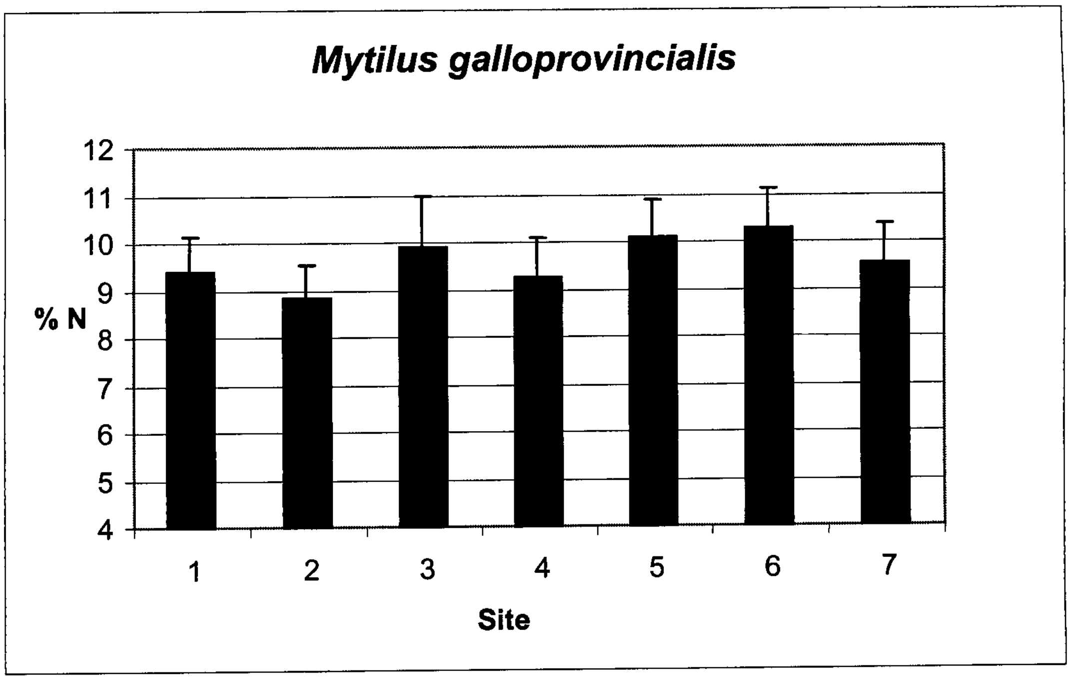


Fig 10: Bar graph showing the average % Nitrogen of *Mytilus galloprovincialis* at each sample site. Data are means ± 1 SE.

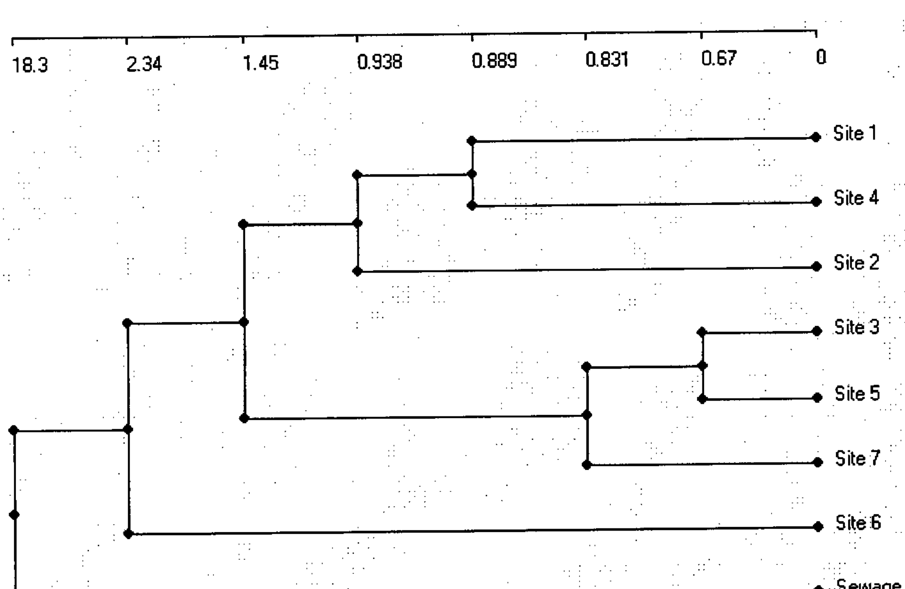


Fig 11: Cluster analysis of $\delta^{15}N$ values of *Mytilus galloprovincialis* at sampling sites using complete linkage and Euclidean distance.

Discussion

In this study the effects of the sewage derived nutrients from the Green Point Outfall on a macroalga (*Ecklonia maxima*) and a filter-feeder (*Mytilus galloprovincialis*) were evaluated using carbon and nitrogen stable isotope analysis. The variables investigated ($\delta^{15}N$ and $\delta^{13}C$) were affected differently by inputs of organic material. Overall $\delta^{13}C$ values of both *Ecklonia maxima* and *Mytilus galloprovincialis* varied little between locations, while the range of $\delta^{15}N$ variation was much greater. As a consequence and in agreement with the literature (McClelland & Valiela, 1998; Heikoop *et al.*, 2000; Risk & Erdmann, 2000), $\delta^{15}N$ is a better indicator than $\delta^{13}C$ of organic enrichment and uptake of anthropogenic material within coastal food webs. The $\delta^{15}N$ isotopic characteristics of *Ecklonia maxima* and *Mytilus galloprovincialis* from near and around Green Point indicate that they are closely linked to source material (urea and ammonia from the sewage) from the outfall.

Ecklonia maxima

The isotopic $\delta^{13}C$ values of *Ecklonia maxima* at all but one (site 5) of the 7 sample sites are slightly enriched relative to our control value (Fig 4). The reasons for this enrichment could be a result of increased primary productivity often associated with nitrogen-rich environments, or a lower availability of dissolved inorganic carbon in these areas (Rogers, 1999). A number of studies have attributed this enrichment to particulate organic material which is derived from sewage and deposited close to the coast; this material may be oxidized and promote eutrophic conditions (Rogers, 1999). These conditions in turn could lead to $\delta^{13}C$ enrichment due to the preferential utilization of the ^{12}C isotope during oxidation reactions (Rogers, 1999). Therefore *Ecklonia maxima* within a sewage contaminated area would have more enriched $\delta^{13}C$ values relative to populations in non-contaminated areas. Site 5 is an interesting exception to this trend as it is situated close to the out fall and has $\delta^{13}C$ values which are depleted and similar to the control compared to the rest of the sites (Fig 4). A possible reason for this is the fact that site 5 runs parallel with the dominant wind and swell direction in this region. It therefore may not accumulate as much sediment as the other sites thereby reducing the effects of eutrophication and resulting in less enriched $\delta^{13}C$ values. Another explanation could be the fact that effluent plumes are known to have a buoyant stage, whereby they are carried by surface currents before becoming fully mixed with the water column (Lord & Hillman 1995). Garther *et al.* (2002) showed that an effluent plume can stay buoyant for 500m beyond the outfall. Site 5 is situated only 400m from the outfall. It is therefore possible that sewage from the outfall is affecting organisms at site 5; however there is no accumulation of sediment and therefore $\delta^{13}C$ values are less enriched relative to sites accumulating sewage sediments.

Moving away from the outfall there is a distinct pattern of increasing $\delta^{15}N$ values with increasing distance from the outfall (Fig 2). This pattern indicates that $\delta^{15}N$ values of *Ecklonia maxima* within contaminated areas are closely linked to the contaminant material (sewage) and will have isotopic values which are depleted relative to the control site where populations assimilated only naturally derived marine nitrate [NO_3^-]. At site 6 located on the outfall *Ecklonia maxima* exhibited isotopic values (1.2±2.3‰) which were very similar to the raw sewage (0.4±0.4‰) which is discharged (Fig 6). $\delta^{15}N$ values show a steady increase from site 6-2 thereby indicating the dominant direction of movement by effluent is south west. This is in agreement with findings by Fijen & Marsden (1992) who determined that because there is not a well defined current system in the Green Point area and the tidal range is small, surface currents in the discharge area are almost completely wind dominated, with the dominant wind direction being SW. Green Point was originally chosen as a site for a marine outfall due to the sites prevailing long shore currents (65% of the time) and the infrequent onshore current occurrence (5% of the time), this confirms the main effluent plume from the outfall will move in a southwesterly direction along the coast (Fijen & Marsden 1992). Site 1 does not however follow this trend as there is a sudden decrease in $\delta^{15}N$ at this site (Bantry Bay) (Fig 2). The reason for this sudden decrease is that when sampling sites were being selected, site 1 was intended to be far enough away from the outfall to act as a control. However after collection and analysis of the samples it was discovered that there was in fact a second sewage outfall situated at Camps Bay only a 1 km away from site 1. Therefore samples at this site may also be contaminated and therefore more depleted relative to sites situated at an intermediate distance from the Green Point outfall (sites 2, 3, 4 and 7). Site 7 also tends to indicate that the dominate direction of movement by effluent from the Green Point outfall is south west. The reason we can assume this is due to the fact that site 7 is situated close to the outfall but does not indicate a large amount of contamination and is similar to site 2 (Fig 6) rather than site 5 as expected. Algae collected from site 7 could however also be contaminated by other nitrogen sources originating from within Table Bay and may therefore be affected by additional inputs not originating from sewage such as power station cooling water, industrial effluents and general inputs from the harbour and ships. These contaminants are unknown and have not been measured for $\delta^{15}N$ values, therefore we cannot make any sound assumptions about what exactly is affecting site 7.

We expected that sites closer to the outfall would have a higher percent nitrogen compared to sites situated further away, because *Ecklonia maxima,* in areas of increased nutrient concentrations would incorporate more nitrogen into the tissue. However as can be seen in Figure 5 this is not the case as site 6 does not have the highest %N, but rather site 7 which is situated on the eastern side of the outfall within Table Bay. Due to the very small range of values it has been proposed that *Ecklonia maxima* in this area may be saturated in terms of N and therefore not show significant differences between sites. The very large value recorded at site 7 may be a result of other nitrogen sources from within Table Bay (Fig 5). These other sources may have different properties to that of sewage and therefore affect the %N within the organism's tissue differently.

Mytilus galloprovincialis

The depletion of ^{13}C in the mussel tissue from specimens sampled in close proximity to the outfall, compared to the control site, is consistent with a contribution of depleted sewage derived organic matter to their diets. Site 6, situated closest to the outlet, showed the largest decrease in $\delta^{13}C$ values', with sites 1 and 5 showing similar depleted values (Fig 11) 8. The reason we are able to see a change in the $\delta^{13}C$ values of mussels in contaminated areas and not kelps is due to the fact that mussels are filter-feeders and are capable of assimilating particulate sewage-derived organic matter directly (Rogers, 2003), whereas macro-algae rely on photosynthesis for their carbon needs. Mussels however are unlikely to depend on a single food source and rely instead on the changing sources of carbon available within the water column, and filter food selectively (Hill *et al.*, 2006). A large number of studies conducted on the Benguela subtidal kelp-bed ecosystems, have consistently shown that much of the detrital material in suspension in shallow waters consists of fragmented kelp, even in coastal areas which lack kelp (Stenton-Dozey & Brown, 1992; Bustamante & Branch, 1996). Therefore it can be expected that mussels in contaminated sites will incorporate both sewage and fragmented kelp into their diets, and their isotopic signatures will therefore reflect a combination of both food sources. My results show that *Mytilus galloprovincialis* relies on kelp for most of its carbon requirements as we can see in Fig 9 that $\delta^{13}C$ values for the 7 sample sites are only 3 ‰ more depleted than our *Ecklonia maxima* control (Fig 9). According to the literature, C isotope signals measured in animals are 0.5 to 1‰ more depleted than their food source (Smit, 2001). Therefore we can assume due to the much larger depletion in $\delta^{13}C$ values that mussels in contaminated areas are incorporating sewage derived organic matter into the tissue. It is also however important to remember that *Ecklonia maxima* in contaminated areas will also have a depleted $\delta^{13}C$ value, and this may also affect the isotopic signatures of mussels which incorporate this kelp.

In Fig 9 we can see that the $\delta^{15}N$ values for mussels vary only slightly, however there is quite a distinct difference between the 7 sample sites and the control.

This trend seems to indicate that mussels in contaminated areas (all sample sites) are incorporating sewage derived N into their tissues. Site 6 has the most depleted $\delta^{15}N$ value as it is situated on the outfall, meaning mussels in this area can filter large amounts of fairly undiluted sewage from the water column. Rogers (1999) showed that mussels sampled in a sewage-contaminated area in New Zealand had a gut content which contained 50% C and 91% N which was derived from sewage. This implies that at least half of the mussel's diet is attributed to sewage derived matter in contaminated areas. As we get further from the outfall we can see that the $\delta^{15}N$ values of specimens collected at the sample sites increase. This is expected as we are moving further from the point of pollution. Site 1 however, as explained earlier, is situated just 1km from the Camps Bay outfall and so is affected by it, rather than the Sea Point outfall, and this is why its values are so similar to site 6.

Site 5 is an interesting one as it does not follow the same trend as the rest of the sites. The reason for this as mentioned before could be due to this site being orientated parallel to the dominant swell direction in this area. This may therefore result in less particulate organic matter reaching mussels close to the shore. Another explanation for the disrupted trend in $\delta^{15}N$ could be due to site 4 as it has more depleted $\delta^{15}N$ values than site 5. The reason for this could be due to the fact that site 4 is more exposed. The reason for its depleted $\delta^{15}N$ values are that exposed sites have a higher concentration of particulate food, as well as a higher water turnover rate that will replenish food more rapidly than on sheltered shores (Bustamante & Branch, 1996). This means that on more exposed shores mussels have greater access to food, which in turn will result in them incorporating more sewage-derived material into their tissue than mussels on sheltered shores. Site 7 is again the most enriched site and further indicates that the dominant water movement in the area is south-west. We can assume this as effluent from the outfall has been shown not to be contaminating site 7. However site 7 may also be affected by other nitrogen inputs from within Table Bay, which are unknown and therefore have not been measured.

In Fig 9 there is a data point for phytoplankton, in order to determine whether they are an important part of the sample mussels' diets. The data suggest that

Acknowledgements

Thanks to Prof John Bolton, Dr Rob Anderson and Dr Edmund February for there knowledgeable insight and help with data interpretation. Special thanks to Guy Brown who braved the swell of Green Point to help me collect samples.

References

- Bartlett, P.D, Henning, HF-KO & Eagle, G.A., 1985. Table Bay – A marine chemical study. CSIR Report 565, Stellenbosch, South Africa
- Bustamante, R.H. & Branch, G.M., 1996. The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. *J. Exp. Mar. Biol & Ecol.* **Vol.196,** pp. 1-28
- Costanzo, S.D., O'donohue, M.J., Dennison, W.C., Loneragan, N.R. & Thomas, M., 2001. A new approach for detecting and mapping sewage impacts. *Mar. Poll. Bulletin* **Vol. 42,** pp. 149-156
- Costanzo, S.D., Udy,J., Longstaff, B. & Jones, A., 2005. Using nitrogen stable isotope ratios ($\delta^{15}N$) of macroalgae to determine the effectivness of sewage upgrades: changes in the extent of sewage plumes over four years in Moreton Bay, Australia. *Mar. Poll. Bulletin* **Vol. 51,** pp. 212-217
- Fijen, A.P.M. & Marsden, M.G., 1992. Green Point Sewage disposal options. *Wat. Sci. Tech.* **Vol. 25,** pp. 133-142
- Gartner, A., Lavery, P. & Smit, A.J., 2002. Use of $\delta^{15}N$ signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage. *Mar. Ecol. Progress series.* **Vol. 235,** pp. 63-73
- Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology.* **Vol. 59,** pp. 87-102
- Hill, J.M., McQuaid, C.D. & Kaehler, S., 2006. Biogeographic and nearshore-offshore trends in isotope ratios of intertidal mussels and their

food scources around the coast of southern Africa. *Mar. Ecol. Progress Series,* **Vol. 318,** pp. 63-73

- Lord, D. & Hillman, K., 1995. Perth coastal waters study: summary report. Water Authority of Western Australia, Leederville, Western Australia
- Peterson, B.J. & Howarth, R.W., 1987. Sulfur, carbon and nitrogen isotopes used to trace organic matter flow in salt-marsh estuaries of Sapelo Island, Georgia. *Liminology and Oceanography,* **Vol. 32** pp. 1195-1213
- Piola, R.F., Moore,S.K. & Suthers, I.M., 2006. Carbon and nitrogen stable isotope analysis of three types of oyster tissue in an impacted estuary. *Eatuarine, Costal and Shelf Science.* **Vol. 66,** pp. 255-266
- Monteiro, P.M.S, 1993. An assessment of the environmental impact of sewage derived organic matter from Green Point Outfall on the sediments of Table Bay using the stable isotope ratio of Nitrogen $\delta^{15}N$. Marine pollution division, Sea Fisheries Research Institute.
- Rogers, M.R., 1999. Effects of sewage contamination on macroalgae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes. *NZ journal of Mar. and Fresh water research.* **Vol. 33**, pp. 181-188
- Rogers, M.R., 2003. Stable carbon and nitrogen isotope signatures indicate recovery of Moa Point, New Zealand. *Mar. Poll. Bulletin* **Vol 46,** pp. 821-827
- Savage, S. & Elmgren, R., 2004. Macroalgal (*Fucus vesiculosus)* $\delta^{15}N$ values trace decrease in sewage influence. *Ecol. Application,* **Vol. 14**(2), pp. 517-526
- Schoeninger, M.J. & Wada, N.J., 1989. Nitrogen and carbon isotope composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta,* **Vol. 48,** pp. 625-639
- Smit, A.J., 2001. Source identification in marine ecosystems: food web studies using $\delta^{13}C$ and $\delta^{15}N$. The University of Western Australia.
- Spies, R.B., Kruger, R.I., Ireland, R. & Rice, D.W., 1989. Stable isotope ratios and contaminant concentrations in a sewage distorted food web. *Mar. Ecol. Progress series.* **Vol. 54**, pp. 157-170

- Stenton-Dozey, J. M. E., Brown. A. C. (1992). Clearance and retention efficiency of natural suspended particles by rock-pool bivalve Venerupis corrugatus in relation to tidal availability. *Mar. Ecol. Prog. Ser.* **Vol.82,** pp. 175-186
- Sweeney, R.E., Kalil, E.K., Kaplan, I.R., 1980. Characterisation of domestic and industrial sewage in southern California coastal sediments using nitrogen carbon, sulphur and uranium tracers. *Marine Environmental Research* **Vol. 3,** 225–243.
- Van Dover, C.L., Grassle, J.F., Fry, B., Garritt, R.H., Starczak, V.R., 1992. Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature* **Vol. 360**, 153–156.
- Waldron, S., Tatner, P., Jack, I. & Arnott, C., 2001. The impact of sewage discharge in a marine embayment: Stable isotope reconnaissance. *Eatuarine, Costal and Shelf Science.* **Vol. 52,** pp. 111-115